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Morphological and cytogenetic characterization in experimental hybrid *Aloe jucunda* Reyn. x *Aloe vera* (L.) Burm. f. (Asphodelaceae)

Wendy Ozols-Narbona*, José Imery-Buiza

Departamento de Biología, Universidad de Oriente, Cumaná, 6101, Venezuela
*Correspondign author. E-mail: wozolsnarbona@gmail.com

Abstract. *Aloe* L. includes plants of economic interest worldwide for their medicinal properties and ornamental character. In this study, morphological and cytogenetic traits were evaluated in a hybrid obtained using *Aloe jucunda* Reyn. as pollen donor and *A. vera* (L.) Burm. f. as female parent, to characterize it, determine its ornamental and agronomic potentialities and aspects related to its reproduction. Conventional protocols for morphometric studies and cytogenetic analysis described for succulent plants were applied. Progeny showed intermediate expressiveness in most of the characteristics, except in the colour of the leaves and flowers (hybrid = *A. jucunda*), as well as for the length of teeth, number, and area of leaf spots and angle between continuous leaves, where it surpassed the expression of both parents, giving it a high ornamental value. The length, width, and thickness of the leaves improved with respect to the paternal genome, so its potential for the exploitation of the gel and latex of its leaves cannot be ruled out. Root tip cells showed a karyotype 2n = 2x = 14 = 8L + 6S = 1L(smsat) + 1L(sm) + 3L(st) + 3L(smsat) + 1S(m) + 5S(sm). Microsporogenesis showed chromosomal abnormalities in 47.4% of the meiocytes, the most frequent being micronuclei in prophase-I, sticky chromosomes in metaphase-I, one or two dicentric bridges accompanied or not by acentric fragments in anaphase-I, -II, and telophase-I, - II, as well as one or two additional microspores. These abnormalities reduce the fertility of their pollen grains and limit their sexual reproduction, providing a better explanation for their sterility.

Keywords: *Aloe*, hybrid, morphological attributes, karyotype, microsporogenesis.

INTRODUCTION

Manual hybridization in plants has aroused interest in the genetic improvement of plants that represent crops of economic interest worldwide (Marasek-Ciolakowska et al. 2018). Among these plants are those included within the genus *Aloe* L., which comprises about 519 species with variable vegetative characteristics depending on their geographical location, temperature, fertility conditions, and availability of water in the soil (Smith and Van-Wyk 2008; MBG 2022). These species are xerophytic and monocotyledonous plants that are characterized by being perennial herbs, shrubs or small trees

with thick roots and rosette-shaped leaves (Carter 1994) with succulent tissues of economic importance for their ornamental attributes and therapeutic uses (Rowley 1997, Imery 2011).

Aloe vera (L.) Burm. f. (=*A. barbadensis* Mill.) is native to the Arabian Peninsula and now cultivated in several warm climatic zones of world including Asia, America, and Europe (Grace et al. 2015, Giannakoudakis et al. 2018). The *A. vera* industry has expanded throughout the world and the mucilaginous gel from the parenchymatous cells in the inner leaf pulp is used in many products, including fresh gel, juice, and multiple formulations for health, medicinal, and cosmetic purposes (Saleem et al. 2022). Its leaf extracts are rich in nutrients and contain over 200 active compounds including simple/complex polysaccharides, amino acids, proteins, enzymes, terpenoids, flavonoids, saponins, minerals, vitamins, phenols, and other metabolites, allowing a broad spectrum of medicinal applications (Saniasiaya et al. 2017). Other industrial perspectives also include the use of *A. vera* derivatives as corrosion inhibitors (Singh et al. 2016), obtaining biodiesel (Silva et al. 2015), growth enhancer (El Sherif 2017), germination accelerator and root development stimulant (Tucuch-Haas et al. 2022), post-harvest coating treatments (Farina at al. 2020), improvement of the swelling capacity of commercial acrylic hydrogels (Guancha-Chalapud et al. 2022), nanobiotechnologies (Arshad et al. 2022, Song et al. 2022), decreased methane release in dairy cows (Singh et al. 2021), bioremediation (Giannakoudakis et al. 2018), among others. The global *A. vera* extracts market value is projected to increase from USD 2,454.5 Million in 2022 to USD 5,153.71 Million by 2032, showing opulent growth of 7.7% (FMI 2022).

On the other hand, *Aloe jucunda* Reyn. evolved separately further south, in the Somali desert, and it is considered an exclusively ornamental plant due to its small size, adaptability, and beauty of its bright green variegated leaves and pink pendant flowers (Reynolds 1950). *A. vera* and *A. jucunda* only coexist in botanical gardens, nurseries, or research laboratories, both species present reproductive barriers such as protandry and self-incompatibility, which is why these plants propagate asexually (Imery and Cequea 2008). However, in reciprocal crosses trials, Imery (2011) obtained viable progeny, using *A. jucunda* as the donor species for the pollen grains and *A. vera* as the female parent. The need to obtain information as a contribution to scientific knowledge about a completely unpublished genotype not yet described, led to the realization of this work, which aimed to evaluate morphological and cytogenetic traits that would allow characterizing this experimental hybrid.

MATERIALS AND METHODS

Vegetal material

A. jucunda and *A. vera* adult plants (over eight years old) growing in the germplasm bank of succulent species of the Biology Department from Universidad de Oriente, located at 10°26'32'' N and 64°09'14'' W, in an area of very dry tropical forest in Cumaná city (Venezuela) were used. Specimens of *A. jucunda* (P2) were originally acquired in local nurseries and those of *A. vera* (P1) came from Península de Araya naturalized population, located at 10°34'15'' N and 64°12'08'' W (Albornoz and Imery 2003). Both species were identified considering the morphotaxonomic descriptions of Jacobsen (1955), Carter (1994) and Van-Wyk and Smith (1996). Five specimens of each of the evaluated genotypes were deposited in the IRBR Herbarium. Other fresh specimens are preserved in the already identified germplasm bank.

Morphological evaluation

Following of the morphometric traits were determined in the progeny and their parents (Figure 1): number, length, width, thickness, and volume (VH=π*LH*AH*EH/12) of the leaves (Hernández-Cruz et al. 2002), number and length of leaf teeth, number and area of leaf spots, leaf insertion angle, angle between continuous leaves, number of suckers, and flower colour, according to Imery and Cequea (2012). Ten adult plants of each genotype (*A. jucunda*, *A. vera*, and experimental progeny) were characterized.

Quantitative variables were analysed using ANOVA and LSD tests at $p \leq 0.05$ (Sokal and Rohlf 1979).

Cytogenetic evaluation

Mitotic chromosomes were studied from temporal slide prepared with meristems of root tips collected at 7:30-8:00 a.m., pre-treated with colchicine (0.05% m/v) for 2 h, fixed in Carnoy II solution (5:3:1 ethanol: glacial acetic acid: chloroform) for 30 min, hydrated in distilled water for 10 min, hydrolysed with HCl (1N) for 10 min and 24ºC, rehydrated in distilled water for 10 min, coloured with orcein (2% m/v) lactopropionic (45% v/v) for 4 min and gently squashed (Fukui and Nakayama 1996). Chromosomes according to their size (Stebbins 1971), length of the short arm (Brandham 1971) and centromere position (Levan et al. 1964) were classified. Microsporogenesis was evaluated in flower buds between 3.7-4.3 mm in length, fixed in Carnoy II and

Figure 1. Vegetative traits of the hybrid and its parents. (a) *Aloe jucunda*, (b) hybrid, (c) *A. vera*, (d) cross section in leaves of *A. jucunda* (upper), hybrid (middle), and *A. vera* (lower), (e) leaf lengths in the three genotypes, (f) contrasting details in the colour of the leaves, spots, and foliar teeth of the three genotypes evaluated, g) differences in the number of leaf spots between the adaxial face and the abaxial face in the leaves of the experimental hybrid, vegetative (h) and floral (i) details of the experimental hybrid. Scale bars = 2 cm.

staining the content of an anther with lactopropionic orcein (Alcorcés et al. 2012). At least five flower buds in meiosis for each genotype were analysed. All slides were systematically evaluated using a Nikon LABPHOT-2 microscope.

Photomicrographs at 400 and 1000 X with a Sony 7.2 digital camera were captured and the images on a computer using the PhotoImpact and SigmaScan Pro 5 programs were examined. Karyological data (chromosomal length, relative length, and long/short arm index) for ANOVA between genotypes and "t-student" tests between homologous chromosomes were used.

Viability of pollen grains

Fertility of the experimental hybrids was estimated by means of the *in vitro* germination of the pollen grains and pollen tube growth according to Sunderland and Roberts (1977) in culture medium with nutrient agar (6 $g.l^{-1}$) and sucrose (0.125 $mol.l^{-1}$), previously standardized for this genotype. Culture medium was autoclaved at 15 PSI for 15 min and five drops were added to ten slides. It was allowed to gel (10 min, 25ºC) and then the pollen grains of flowers kept in a humid chamber were dispersed until anthesis. Observation was carried out in a Nikon optical microscope, model LABPHOT-2 at 100X, after 60 min in the 10 slides of the microcultures established and covered by a Petri dish to avoid desiccation. For a better contrast, two drops of Astra Blue were added to each slide to colour the pollen tubes (Danti et al. 2011). A viable pollen grain was expected when the length of the pollen tube was greater than or equal to the length of its polar axis (Kalinganire et al. 2000). The percentage of *in vitro* germination of pollen grains was estimated using the relationship between the number of germinated pollen grains and the total number of pollen grains contained in each microscopic field.

RESULTS AND DISCUSSION

Genotypes evaluated (P1: *A. vera*, P2: *A. jucunda*, and H: hybrid) were significantly different ($p \leq 0.05$) in all the morphological variables studied. In most of the characteristics, the progeny was expressed in an intermediate way between its parents, except in the colour of the leaves, flowers and the presence of spots, which were inherited from the paternal genome of *A. jucunda*, as well as for the length of the teeth, angle between continuous leaves, number of spots on the adaxial side and number and area of leaf spots on both the adaxial and abaxial sides, in which the hybrid exceeded the expression of both parents (Table 1). The bright green colour and variegated character of its leaves thanks to the presence of spots because of the contribution of the paternal genome of *A. jucunda*, give this hybrid a high ornamen-

Table 1. Morphological attributes evaluated in adult plants of the progeny and their parents *Aloe jucunda* and *A. vera*, under nursery conditions in Cumaná (Venezuela).

Attribute/Genotype	A. vera (P1)	A. jucunda (P2)	Hybrid (H)	P1/P2	H/P1	H/P2
Leaf colour	Grey-green	Pine-green	Pine-green	-	-	-
Number of leaves	24.90 ± 2.85^{a}	18.90 ± 3.38^{c}	23.40 ± 2.22^{b}	1.32	0.94	1.24
Leaf length (cm)	57.41 ± 5.24^{a}	7.19 ± 0.28^{c}	28.28 ± 3.27^{b}	7.98	0.49	3.93
Leaf width (mm)	74.79 ± 5.31^{a}	15.74 ± 1.40^{c}	35.61 ± 4.64^{b}	4.75	0.48	2.26
Leaf thickness (mm)	21.74 ± 2.34^{a}	9.42 ± 0.83^{c}	17.82 ± 1.63^{b}	2.31	0.82	1.89
Leaf volume (cm^3)	244.39 ± 41.21^{a}	2.81 ± 0.45^{c}	47.47 ± 11.48^{b}	86.97	0.19	16.89
Leaf insertion angle (°)	31.71 ± 2.93^{c}	79.06 ± 7.30^{a}	38.44 ± 5.78^{b}	0.40	1.21	0.49
Angle between leaves (°)	80.90 ± 2.99^{c}	105.37 ± 19.15^{b}	124.72 ± 14.64^{a}	0.77	1.54	1.18
Number of leave teeth	35.08 ± 1.05^{a}	20.30 ± 1.37^{c}	23.43 ± 1.84^{b}	1.73	0.66	1.15
Teeth length (mm)	2.75 ± 0.22^{b}	1.38 ± 0.20^{c}	3.61 ± 0.47^{a}	1.99	1.31	2.62
Number of spots (adaxial)	0.00 ± 0.00^{c}	51.13 ± 6.06^{b}	59.27 ± 19.69^{a}	0.00	∞	1.16
Number of spots (abaxial)	0.00 ± 0.00^{c}	225.37 ± 15.65^{a}	194.37 ± 28.43^{b}	0.00	∞	0.86
Adaxial spot area (mm^2)	0.00 ± 0.00^{c}	1.82 ± 0.48^{b}	16.35 ± 6.35^{a}	0.00	∞	8.98
Abaxial spot area (mm^2)	0.00 ± 0.00^{c}	0.93 ± 0.27^{b}	15.56 ± 7.59^{a}	0.00	∞	16.73
Number of basal suckers	6.80 ± 2.04^{a}	4.10 ± 0.99^{c}	5.10 ± 3.03^{b}	0.75	0.63	1.24
Flower colour	Yellow	Orange-pink	Orange-pink	-	-	-

Values indicate mean ± standard deviation with n = 10 plants. Numbers followed by same letter are not significantly different (LSD $p \leq 0.05$) between genotypes.

tal value. Traits such as the length, width, thickness, and volume of the leaves were significantly improved in the progeny because of the contribution of the maternal genome (*A. vera*). In these cases, the magnitude of the improvements was between 1.24 to 16.89 times higher than the expression of the parent *A. jucunda* (smaller parent), so its possible agronomic potential for exploitation is not ruled out both of gel and latex of its leaves (Figure 1).

Another attribute of interest is the increase in the dimensions of the foliar teeth, which gives this new genotype an advantage as a defence mechanism against some predators. Watson et al. (2003) comment that the overexpression of some characteristic in hybrid descendants could be attributed to the accumulation of numerous loci in heterozygosis, propitiated by the interaction of two different genomes, in this case, that intervene in the organogenesis of the spines or biomass leaf to the edges. On the other hand, the prolific vegetative propagation guarantees the hybrid to perpetuate itself over time, compensating for its sterility, since it has not formed fruits and seeds through sexual reproduction, which, according to Imery and Cequea (2008), could be attributed to self-incompatibility mechanisms inherited from the maternal genome of *A. vera*.

Morphometric characterization of the progeny reveals a considerable ornamental value in this new genotype and the possibility of incorporating it as a model for studying the inheritance of traits of ornamental value and/or agronomic importance or for future crosses in the search for new genotypes, and complementary research.

Root tip meristematic cells presented bimodal karyotypes and chromosomal classifications (Levan et al. 1964) described by the formulas $2n = 2x = 14 = 8L + 6S = 2L(smsat) + 4L(st) + 2L(stsat) + 2S(m) + 4S(sm)$ in *A. vera* with eight large chromosomes (L) measuring 13.4-15.5 µm and six small chromosomes (S) measuring 5.1-5.9 µm; $2n = 2x = 14 = 8L + 6S = 2L(sm) + 2L(st) + 4L(stsat) + 5S(sm) + 1S(m)$ in *A. jucunda* with eight L chromosomes (14.7-16.9 µm) and six S chromosomes (5.5-6.3 µm); and $2n = 2x = 14 = 8L + 6S = 1L(smsat) + 1L(sm) + 3L(st) + 3L(stsat) + 1S(m) + 5S(sm)$ in the hybrid with eight L chromosomes (13.8-15.8 µm) and six S chromosomes (5.2-6.2 µm) (Figure 2).

Heteromorphisms between the homologues of chromosome pairs L2 and S1 were determined in the hybrids named VJ6 and VJ10, respectively (Figure 2c,d). As the genotypes evaluated did not show significant differences in the length of each of their chromosomes, the possibility that heteromorphisms between homologues of the progeny are caused by chromosomal mutations such as deletions is ruled out. In this regard, Brandham (1976) evaluated the karyotypes of 1543 diploid plants of the *Aloe*, *Gasteria*, and *Haworthia* genera without finding evidence of deletion. However, in polyploid species of the genus *Aloe* he found a frequency of 5.8%, indicating that structural mutations of this type have a lethal effect in diploid species. That is why heteromorphisms between homologous chromosomes are mainly attributed to the fact that their chromosomal complement comes from two different genomes.

Chromosomal abnormalities were found in 47.4% of the meiocytes evaluated. The most frequent meiotic aberrations were formation of micronuclei in prophase-I, sticky chromosomes, and acentric fragments in metaphase-I and -II, dicentric bridges accompanied or not with acentric or linked fragments in anaphase-I and -II, occasionally persistent in prophase-II, asynchrony between telophase-I and prophase-II, bridges, fragments, and micronuclei in telophase-I and -II, one or two additional microspores of variable size at the end of microsporogenesis (Figure 4). Although 31.6% of the pollen grains evaluated in the present investigation germinated under *in vitro* conditions (Figure 5), the absence of fruits and seeds in this new genotype forces this plant to depend exclusively on vegetative propagation for its multiplication.

Reproductive barriers such as gametophytic and sporophytic self-incompatibility have been described for most species of the *Aloe* genus (Newton 2004), including *A. vera* (Imery and Cequea 2008) and *A. jucunda* (Riley and Majumdar 1979), limiting then its self-fertilization with those pollen grains not affected by microsporogenic irregularities. This leads to the deduction that the impossibility of sexual reproduction of the experimental hybrids is also related to incompatibility genes inherited from both parents.

Swamy and Krishnamurthy (1980) and Imery (2011), argue that many plant species are forced to propagate asexually due to the existence of chromosomal alterations (deletions, inversions, and translocations), transmitted from their parents, either because they were present in their genomes or because they originated during the formation of their sex cells.

Additional bridges, fragments, micronuclei, and microspores are frequent in *Aloe* species with heterozygous paracentric inversions (Riley and Majumdar 1979, Ahirwar and Verma 2013). The pairing between the inverted chromosome and its pachytene homologue must involve the formation of a loop where crossovers occur between homologous chromatids that generate fine chromatin threads linked to two centromeres and totally unlinked fragments of these, causing the loss of

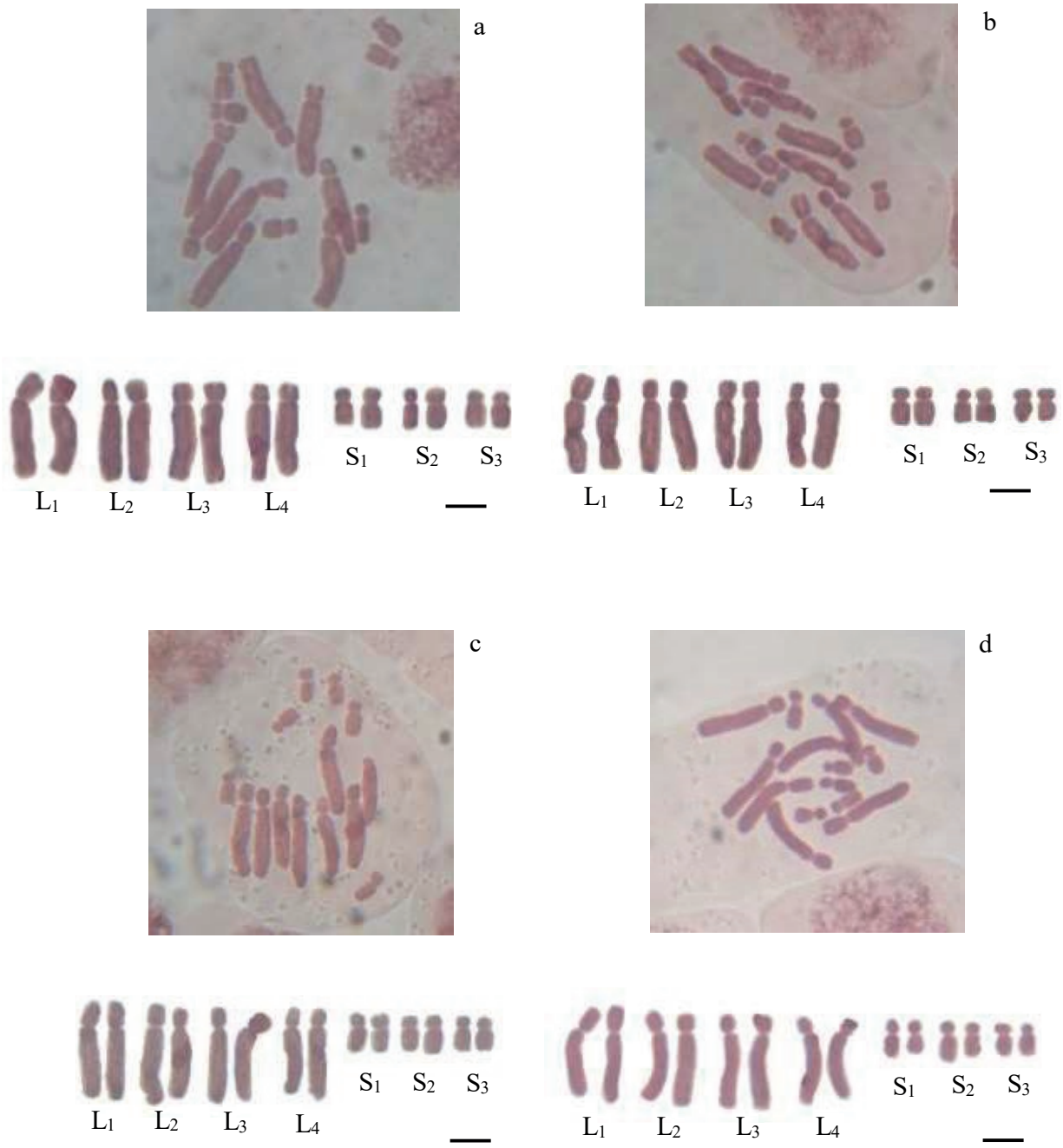

Figure 2. Mitotic chromosomes in root tip cells and bimodal karyograms (2*n*=2x=14=8L+6S) of a) *Aloe jucunda*, b) *A. vera*, c, d) experimental hybrids with greater heteromorphisms between homologous chromosomes. Typing of large (L) and small (S) chromosomes according to Brandham (1971). Scale bars = 5 µm.

genes that reduce the fertility of gametes. Chromosomal fragments present individually or linked to dicentric bridges during anaphase-I or telophase-I generally form additional micronuclei that increase the number of microspores at the end of meiosis and cause gene deficiencies (Ahirwar and Verma 2013). These aberrations may be present in clones that were formed by vegetative propagation from carrier individuals (populations of *A. vera* from Eastern Venezuela) and may have been inherited by the progeny or may be generated during the formation of sexual cells (Cequea et al. 2003, Imery 2011).

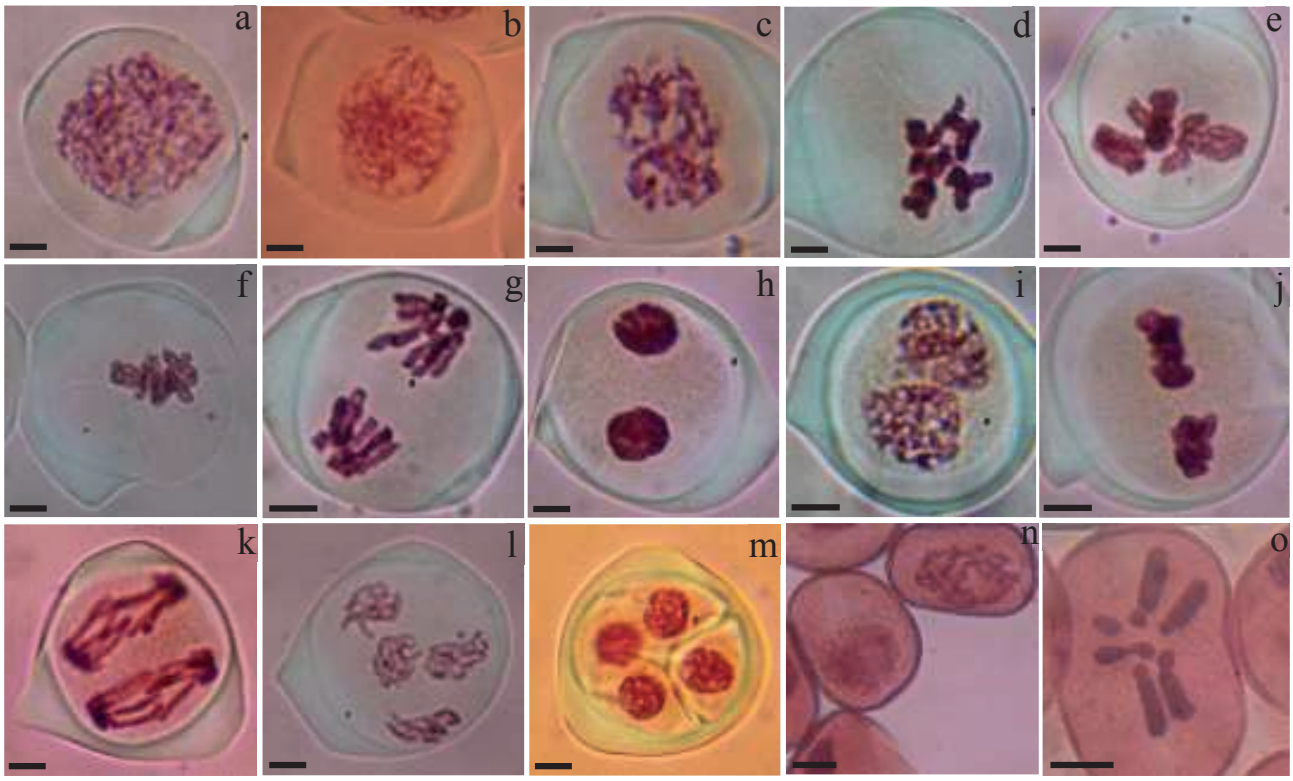

Figure 3. Microsporogenesis and microspore mitosis of the experimental hybrid *Aloe jucunda* x *A. vera*. a) Prophase-I (Leptotene), b) Prophase-I (zygotene), c) Prophase-I (pachytene), d) Prophase-I (diplotene), e) Prophase-I (diakinesis), f) Metaphase-I , g) anaphase-I, h) telophase-I, i) prophase-II, j) metaphase-II, k) anaphase-II, l) telophase- II, m) tetrad, n) microspores initiating first mitosis, o) microspore in metaphase showing haploid chromosomes (n=x=7=4L+3S). Scale bars = 10 µm.

On the other hand, Baptista et al. (2000) mention that the high frequency of sticky chromosomes or agglutination could be due to a genetic-environmental interaction associated with high temperatures, causing chromatin instability mainly in metaphase-I. Sapre (1975) suggests that the participation of neocentric activity in early displacement of smaller chromosomes is the main cause of dicentric bridges between large homologues; however, Imery and Cequea (2002) attributes this to early dissolution of the synaptonemal complex between small homologous chromosomes.

Other causes that have been mentioned to explain the presence of failures during meiosis are environmental conditions. Palmer et al. (2000), obtained discrepancies in the percentage of abnormalities between *Glycine max* plants that grew in different environmental conditions. These authors argue that the plants analysed grew in two contrasting environments and that the high temperatures increased the frequency of meiotic aberrations. A cytogenetic study in *Abies sibirica* (gymnosperm) conducted by Bazhina et al. (2008) revealed the same trend, noting that temperature fluctuations in the different months of the year affected the frequency of abnormalities. In this case, the plants evaluated during the dry season of summer registered a greater number of anomalies with respect to those analysed in the cool season of spring. Imery (2011) points out the possibility that the environmental conditions associated with high temperatures, solar radiation, and low humidity could promote the increase in the concentration of some substances typical of the plant (anthrones, anthraquinones) that alter the normal division of pollen mother cells in *A. vera*. It is possible, then, that the high environmental sensitivity and the existence of structural mutations already reported in *A. vera* were inherited to their sexual descendants *A. jucunda* x *A. vera* explain the origin of the abnormalities observed in this investigation.

Failures in the union of the kinetochores to the meiotic spindle could explain the presence of lagging or asynaptic chromosomes in metaphase and anaphase-I and -II, causing an independent behaviour of the rest of the chromosomes that make up the nucleus (Ishii and Akiyoshi 2022), and the depolymerization of spindle at different times in each cell nucleus could be the reason

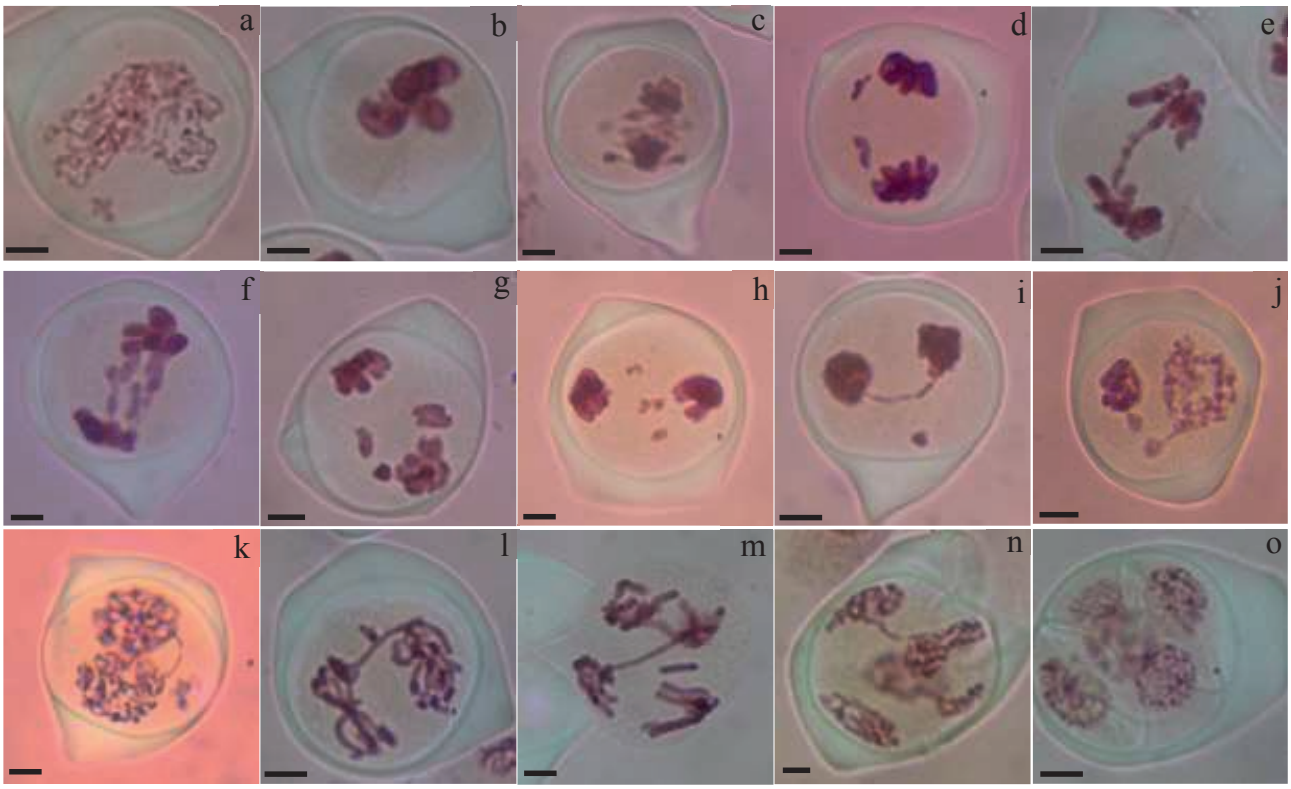

Figure 4. Most frequent meiotic abnormalities in the hybrid *Aloe vera* x *A. jucunda*. (a) Micronucleus in prophase-I; (b-c) sticky chromosomes and early displacement of small chromosomes in metaphase-I; (d-h) acentric fragments in anaphase-I and telophase-I; (e-f) one and two dicentric bridges in anaphase-I; (g) lagging chromosomes in anaphase-I; (i-k-n) bridge and fragment in telophase-I, -II and prophase-II; (j) phase asynchrony between telophase-I and prophase-II; (l) bridging and metaphase-II fragment; (m) bridge and fragment in anaphase-II and (o) additional microspore at the end of microsporogenesis. Scale bars = 10 µm.

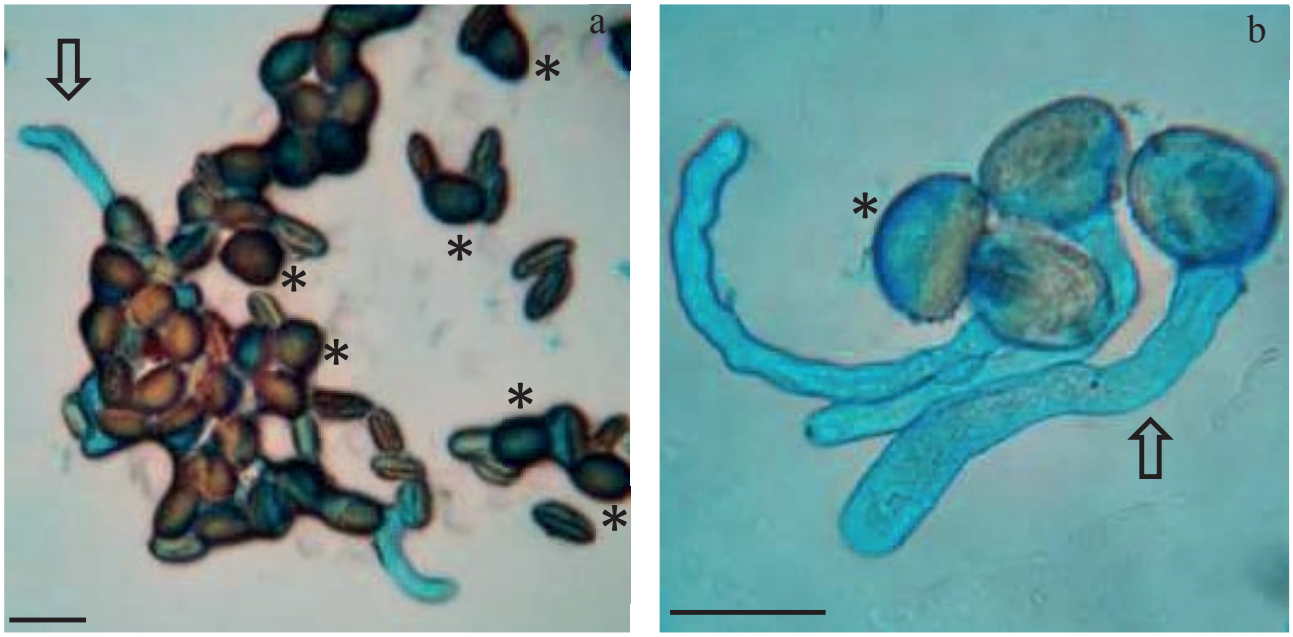

Figure 5. Pollen grains of the experimental hybrid *Aloe jucunda* x *A. vera*. Viability test of pollen cultured in vitro with agar-sucrose medium. Arrows point to pollen tubes of germinated pollen grains considered viable, while the (*) indicate non-germinated or non-viable pollen grains. Scale bars = 50 µm.

for the phase asynchrony between telophase-I/prophase-II and anaphase-I/telophase-II (Alcorcés et al. 2007).

CONCLUSIONS

Experimental hybrids of *Aloe vera* x *A. jucunda* showed superiority of vegetative traits such as the length of the foliar teeth, angle between continuous leaves, number and area of the spots compared to their parents, conferring them a high ornamental value. Traits such as the length, width, thickness, and volume of the leaves improved considerably with respect to the paternal genome, so its possible agronomic potential for the exploitation of the gel and latex of its leaves cannot be ruled out. Root tip cells presented the expected bimodal karyotype and number of chromosomes for the species of this genus. The meiotic abnormalities present in the progeny decrease the fertility of the pollen grains and show the reasons for their limited sexual reproduction, providing a better explanation for the absence of fruits and seeds in all their flowering periods.

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